Biochemistry (Moscow) 1997 vol.62 N8, pages 903-908

Glutamyl endopeptidase of Bacillus intermedius strain 3-19. Purification, properties, and crystallization

Leshchinskaya I., Shakirov E., Itskovitch E., Balaban N., Mardanova A., Sharipova M., Blagova E., Levdikov V., Kuranova I., Rudenskaya G., Stepanov V.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

A homogeneous glutamyl endopeptidase splitting peptide bonds of glutamic and, rarely, of aspartic acid residues in peptides and proteins was isolated from Bacillus intermedius 3-19 culture filtrate using chromatography on CM-cellulose and Mono S. The enzyme molecular mass is 29 kD and the p/ is 8.4. The proteinase is inhibited by DFP. The enzyme, like other glutamyl endopeptidases, reveals two pH optima (pH 7.5 and 9.0) for casein and one (pH 8.0) for Z-Gl--pNA hydrolysis. The Km for the hydrolysis of the latter substrate is 6 mM. The enzyme activity is optimal at 55°C. The enzyme is stable in the pH range 6.5-11.0. Its N-terminal sequence shows 56% coinciding residues when compared with that of Bacillus licheniformis glutamyl endopeptidase. Crystal prisms or plates 0.25-0.3 × 0.15 × 0.07-0.1 mm have been grown using the vapor diffusion technique in a hanging drop followed by macroseeding. The crystals belong to the space group B2 with the following unit cell parameters: a = 69.59 Å; b = 61.61 Å; c = 56.11 Å; γ = 117.57°. The X-ray data set to 1.7 Å resolution has been collected on an automatic synchrotron (EMBL Hamburg Station).

Keywords

Crystallization, Glutamyl endopeptidase (Bacillus intermedius), Isolation, Proteases, Specificity